

**CLAIM AMENDMENTS**

Claim 1 (currently amended): A combination against dental caries bacteria, characteristic in comprising effective components composed with IgY prepared from streptococcus mutans type c and type d to dental caries bacteria and ~~an antiseptic composed with~~ at least one of potassium sorbate and sodium benzoate, wherein an additive amount of said IgY is 0.05-0.2% and an additive of one of said potassium sorbate and sodium benzoate is 0.005-0.02%, wherein said IgY is prepared by using said streptococcus mutans type c and type d as antigen comprising the steps of:

(a) immunizing hens with said streptococcus mutans type c and type d mixture;

(b) extracting egg yolk by distilled water and obtaining crude extract;

(c) applying said crude extract on DEAE-Sephadex A50 column; and

(d) applying eluates needed on Sephadex G200.

Claims 2-3 (canceled):

Claim 4 (currently amended): The combination, as recited in claim 3 1, wherein the combination which is a liquid product used for oral cavity is packaged in pocket atomizer for spraying usage.

Claim 5 (currently amended): The combination, as recited in claim 3 1, wherein the combination which is a liquid food is packaged in sucking bottle.

Claim 6 (withdrawn): The combination, as recited in claim 1, wherein the IgY against dental caries bacteria is prepared by the following steps:

(a) preparing streptococcus mutans antigen as antigen bacteria by the steps of;

(a1) separately cultivating the streptococcus mutans type c and type d in a culture medium for 2 to 3 days;

(a2) collecting bacteria by centrifugation;

- (a3) washing the bacteria 4 to 6 times with 0.05-0.2M of phosphate buffered saline, pH 6-7, and heating at 50-60°C for 25 to 35 minutes;
- (a4) mixing the streptococcus mutans type c and type d in a ratio of 2:1; and
- (a5) adding Freund's adjuvant equal to total volume of the streptococcus mutans type c and type d with high speed homogenized;
- (b) immunizing hens with the streptococcus mutans antigen to obtain eggs with active antibody from the hens for 13 months;
- (c) extracting a crude IgY from the eggs by water dilution method;
- (d) applying the crude IgY on "DEAE-Sephadex A50" column and eluting with phosphate buffer containing 0.07M of NaCl to obtain eluates of protein peak;
- (e) applying the eluates of protein peak on Sephadex G200 column and eluting with phosphate buffer containing 0.1M of NaCl to obtain new eluates of protein peak;
- (f) collecting the new eluates of protein peak;
- (g) estimating antibody activity of the eluates of protein peaks with "ELISA";  
and
- (h) eliminating bacteria by 0.22µm membrane filtration and lyophilizing to achieve the IgY against dental caries bacteria.

Claim 7 (withdrawn): The combination, as recited in claim 6, wherein the step (b) comprises the steps of:

- (b1) immunizing the hens by three hypodermic injections of  $1 \times 10^9$ /ml of the streptococcus mutans antigens each time at two weeks intervals;
- (b2) collecting and sterilizing the eggs from 20<sup>th</sup> day after the first hypodermic injection; and

(b3) taking out yolks from the eggs by sieve.

Claim 8 (withdrawn): The combination, as recited in claim 7, wherein the step (c) comprises the steps of:

(c1) evenly stirring the yolks and diluting with 4-6 fold of distilled water to obtain a diluted yolk solution;

(c2) adjusting the diluted yolk solution to pH 4.5-6.5;

(c3) standing the diluted yolk solution at 3-5°C for 20-30 hours;

(c4) centrifuging the diluted yolk solution for 20-30 minutes to obtain a supernatant; and

(c5) concentrating the supernatant by ultrafiltration, eliminating bacteria and lyophilization to achieve the crude IgY.

Claim 9 (withdrawn): The combination, as recited in claim 2, wherein the IgY against dental caries bacteria is prepared by the following steps:

(a) preparing streptococcus mutans antigen as antigen bacteria by the steps of;

(a1) separately cultivating the streptococcus mutans type c and type d in a culture medium for 2 to 3 days;

(a2) collecting bacteria by centrifugation;

(a3) washing the bacteria 4 to 6 times with 0.05-0.2M of phosphate buffered saline, pH 6-7, and heating at 50-60°C for 25 to 35 minutes;

(a4) mixing the streptococcus mutans type c and type d in a ratio of 2:1; and

(a5) adding Freund's adjuvant equal to total volume of the streptococcus mutans type c and type d with high speed homogenized;

(b) immunizing hens with the streptococcus mutans antigen to obtain eggs with active antibody from the hens for 13 months;

(c) extracting a crude IgY from the eggs by water dilution method;

(d) applying the crude IgY on "DEAE-Sephadex A50" column and eluting with phosphate buffer containing 0.07M of NaCl to obtain eluates of protein peak;

(e) applying the eluates of protein peak on Sephadex G200 column and eluting with phosphate buffer containing 0.1M of NaCl to obtain new eluates of protein peak;

(f) collecting the new eluates of protein peak;

(g) estimating antibody activity of the eluates of protein peaks with "ELISA";  
and

(h) eliminating bacteria by 0.22µm membrane filtration and lyophilizing to achieve the IgY against dental caries bacteria.

Claim 10 (withdrawn): The combination, as recited in claim 9, wherein the step (b) comprises the steps of:

(b1) immunizing the hens by three hypodermic injections of  $1 \times 10^9$ /ml of the streptococcus mutans antigens each time at two weeks intervals;

(b2) collecting and sterilizing the eggs from 20<sup>th</sup> day after the first hypodermic injection; and

(b3) taking out yolks from the eggs by sieve.

Claim 11 (withdrawn): The combination, as recited in claim 10, wherein the step (c) comprises the steps of:

(c1) evenly stirring the yolks and diluting with 4-6 fold of distilled water to obtain a diluted yolk solution;

(c2) adjusting the diluted yolk solution to pH 4.5-6.5;

- (c3) standing the diluted yolk solution at 3-5°C for 20-30 hours;
- (c4) centrifuging the diluted yolk solution for 20-30 minutes to obtain a supernatant; and
- (c5) concentrating the supernatant by ultrafiltration, eliminating bacteria and lyophilization to achieve the crude IgY.

Claim 12 (withdrawn): The combination, as recited in claim 3, wherein the IgY against dental caries bacteria is prepared by the following steps:

- (a) preparing streptococcus mutans antigen as antigen bacteria by the steps of;
  - (a1) separately cultivating the streptococcus mutans type c and type d in a culture medium for 2 to 3 days;
  - (a2) collecting bacteria by centrifugation;
  - (a3) washing the bacteria 4 to 6 times with 0.05-0.2M of phosphate buffered saline, pH 6-7, and heating at 50-60°C for 25 to 35 minutes;
  - (a4) mixing the streptococcus mutans type c and type d in a ratio of 2:1; and
  - (a5) adding Freund's adjuvant equal to total volume of the streptococcus mutans type c and type d with high speed homogenized;
- (b) immunizing hens with the streptococcus mutans antigen to obtain eggs with active antibody from the hens for 13 months;
- (c) extracting a crude IgY from the eggs by water dilution method;
- (d) applying the crude IgY on "DEAE-Sephadex A50" column and eluting with phosphate buffer containing 0.07M of NaCl to obtain eluates of protein peak;
- (e) applying the eluates of protein peak on Sephadex G200 column and eluting with phosphate buffer containing 0.1M of NaCl to obtain new eluates of protein peak;

- (f) collecting the new eluates of protein peak;
  - (g) estimating antibody activity of the eluates of protein peaks with "ELISA";
- and
- (h) eliminating bacteria by 0.22 $\mu$ m membrane filtration and lyophilizing to achieve the IgY against dental caries bacteria.

Claim 13 (withdrawn): The combination, as recited in claim 12, wherein the step (b) comprises the steps of:

- (b1) immunizing the hens by three hypodermic injections of  $1 \times 10^9$ /ml of the streptococcus mutans antigens each time at two weeks intervals;
- (b2) collecting and sterilizing the eggs from 20<sup>th</sup> day after the first hypodermic injection; and
- (b3) taking out yolks from the eggs by sieve.

Claim 14 (withdrawn): The combination, as recited in claim 13, wherein the step (c) comprises the steps of:

- (c1) evenly stirring the yolks and diluting with 4-6 fold of distilled water to obtain a diluted yolk solution;
- (c2) adjusting the diluted yolk solution to pH 4.5-6.5;
- (c3) standing the diluted yolk solution at 3-5°C for 20-30 hours;
- (c4) centrifuging the diluted yolk solution for 20-30 minutes to obtain a supernatant; and
- (c5) concentrating the supernatant by ultrafiltration, eliminating bacteria and lyophilization to achieve the crude IgY.

Claim 15 (withdrawn): The combination, as recited in claim 3, wherein the IgY against dental caries bacteria is prepared by the following steps:

(a) preparing streptococcus mutans antigen as antigen bacteria by the steps of;

(a1) separately cultivating the streptococcus mutans type c and type d in a culture medium for 2 to 3 days;

(a2) collecting bacteria by centrifugation;

(a3) washing the bacteria 4 to 6 times with 0.05-0.2M of phosphate buffered saline, pH 6-7, and heating at 50-60°C for 25 to 35 minutes;

(a4) mixing the streptococcus mutans type c and type d in a ratio of 2:1; and

(a5) adding Freund's adjuvant equal to total volume of the streptococcus mutans type c and type d with high speed homogenized;

(b) immunizing hens with the streptococcus mutans antigen to obtain eggs with active antibody from the hens for 13 months;

(c) extracting a crude IgY from the eggs by water dilution method;

(d) applying the crude IgY on "DEAE-Sephadex A50" column and eluting with phosphate buffer containing 0.07M of NaCl to obtain eluates of protein peak;

(e) applying the eluates of protein peak on Sephadex G200 column and eluting with phosphate buffer containing 0.1M of NaCl to obtain new eluates of protein peak;

(f) collecting the new eluates of protein peak;

(g) estimating antibody activity of the eluates of protein peaks with "ELISA";  
and

(h) eliminating bacteria by 0.22µm membrane filtration and lyophilizing to achieve the IgY against dental caries bacteria.

Claim 16 (withdrawn): The combination, as recited in claim 15, wherein the step (b) comprises the steps of:

(b1) immunizing the hens by three hypodermic injections of  $1 \times 10^9$ /ml of the streptococcus mutans antigens each time at two weeks intervals;

(b2) collecting and sterilizing the eggs from 20<sup>th</sup> day after the first hypodermic injection; and

(b3) taking out yolks from the eggs by sieve.

Claim 17 (withdrawn): The combination, as recited in claim 16, wherein the step (c) comprises the steps of:

(c1) evenly stirring the yolks and diluting with 4-6 fold of distilled water to obtain a diluted yolk solution;

(c2) adjusting the diluted yolk solution to pH 4.5-6.5;

(c3) standing the diluted yolk solution at 3-5°C for 20-30 hours;

(c4) centrifuging the diluted yolk solution for 20-30 minutes to obtain a supernatant; and

(c5) concentrating the supernatant by ultrafiltration, eliminating bacteria and lyophilization to achieve the crude IgY.

Claim 18 (withdrawn): The combination, as recited in claim 5, wherein the IgY against dental caries bacteria is prepared by the following steps:

(a) preparing streptococcus mutans antigen as antigen bacteria by the steps of;

(a1) separately cultivating the streptococcus mutans type c and type d in a culture medium for 2 to 3 days;

(a2) collecting bacteria by centrifugation;

(a3) washing the bacteria 4 to 6 times with 0.05-0.2M of phosphate buffered saline, pH 6-7, and heating at 50-60°C for 25 to 35 minutes;



- (a4) mixing the streptococcus mutans type c and type d in a ratio of 2:1; and
- (a5) adding Freund's adjuvant equal to total volume of the streptococcus mutans type c and type d with high speed homogenized;
- (b) immunizing hens with the streptococcus mutans antigen to obtain eggs with active antibody from the hens for 13 months;
- (c) extracting a crude IgY from the eggs by water dilution method;
- (d) applying the crude IgY on "DEAE-Sephadex A50" column and eluting with phosphate buffer containing 0.07M of NaCl to obtain eluates of protein peak;
- (e) applying the eluates of protein peak on Sephadex G200 column and eluting with phosphate buffer containing 0.1M of NaCl to obtain new eluates of protein peak;
- (f) collecting the new eluates of protein peak;
- (g) estimating antibody activity of the eluates of protein peaks with "ELISA";
- and
- (h) eliminating bacteria by 0.22 $\mu$ m membrane filtration and lyophilizing to achieve the IgY against dental caries bacteria.

Claim 19 (withdrawn): The combination, as recited in claim 18, wherein the step (b) comprises the steps of:

- (b1) immunizing the hens by three hypodermic injections of  $1 \times 10^9$ /ml of the streptococcus mutans antigens each time at two weeks intervals;
- (b2) collecting and sterilizing the eggs from 20<sup>th</sup> day after the first hypodermic injection; and
- (b3) taking out yolks from the eggs by sieve.

Claim 20 (withdrawn): The combination, as recited in claim 19, wherein the step (c) comprises the steps of:

(c1) evenly stirring the yolks and diluting with 4-6 fold of distilled water to obtain a diluted yolk solution;

(c2) adjusting the diluted yolk solution to pH 4.5-6.5;

(c3) standing the diluted yolk solution at 3-5°C for 20-30 hours;

(c4) centrifuging the diluted yolk solution for 20-30 minutes to obtain a supernatant; and

(c5) concentrating the supernatant by ultrafiltration, eliminating bacteria and lyophilization to achieve the crude IgY.